Chapter 8
Intranasal Delivery of Drugs for Ischemic Stroke Treatment: Targeting IL-17A

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Abstract Stroke is the second most common cause of death worldwide and a major cause of disability. However, uncertainty surrounds the efficacy and safety of peripheral or intracerebroventricular drug administration for stroke treatment. Intranasal delivery is emerging as a noninvasive option for delivering drugs to the central nervous system with minimal peripheral exposure. Use of the intranasal route could potentially reduce systemic exposure and side effects. Intranasal delivery provides rapid onset that occurs within minutes. Additionally, this method facilitates the delivery of large and/or charged molecules, which fail to effectively cross the blood-brain barrier. We have shown previously that intranasal delivery of exogenous interleukin-17A (IL-17A) promotes the survival, neuronal differentiation, and subsequent synaptogenesis of neural precursor cells in the subventricular zone during stroke recovery, as well as spontaneous recovery and angiogenesis. Therefore, although IL-17A is well-known for contributing to damage in acute ischemic stroke, it might also mediate neurorepair and spontaneous recovery after stroke when delivered intranasally.

Keywords Central nervous system · Interleukin-17 A · Intranasal delivery · Neurorepair · Stroke

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8.1 Introduction

Stroke is the second most common cause of death and a major cause of permanent disability in adults worldwide [1, 2]. Tissue plasminogen activator (tPA) is the only drug approved by the U.S. Food and Drug Administration for thrombolytic therapy after ischemic stroke, but its efficacy and safety are limited by its narrow treatment time window and side effects [3]. In contrast, a broader window exists to promote repair and decrease stroke-associated disability in late phases. Under physiologic conditions, the normal adult brain contains two neurogenic regions: the subventricular zone (SVZ) of the lateral ventricle and the dentate gyrus of the hippocampus [4]. Although stroke induces neurogenesis in the SVZ and the migration of neural precursor cells into the injured striatum [5, 6], the contribution of endogenous neurogenesis to spontaneous recovery after stroke is exceptionally limited, leaving the affected individual with life-long neurologic deficits [7]. Angiogenesis has been shown to be coupled with neurogenesis in brain tissue repair and remodeling after stroke [8]. Therefore, therapeutic interventions are required to promote recovery after stroke by increasing SVZ neurogenesis and angiogenesis.

Traditionally, neurologic disorders, like many bodily disorders, have been treated through peripheral drug administration (predominantly oral administration). However, a variety of disadvantages are associated with using peripheral administration to treat central nervous system (CNS) diseases. Systemic administration can lead to side effects and low bioavailability as a result of first-pass hepatic and intestinal metabolism, plasma protein binding, and the ability of the blood-brain barrier (BBB) to severely restrict entry of all but small, nonpolar compounds. Substantial evidence has shown that intranasal administration represents the most promising, novel, noninvasive method for delivering therapeutic substances directly to the CNS. Here we discuss the advantages of using the intranasal route over peripheral or intracerebroventricular (ICV) routes for treating ischemic stroke. We also introduce our recent study, which showed that intranasal delivery of interleukin-17A (IL-17A) promotes neurogenesis and functional recovery in the later phases of stroke.

8.2 Intranasal Delivery for the Treatment of Neurologic Disorders

The intranasal delivery method was first developed by Frey in 1989 for targeting neurotrophic factors (e.g., nerve growth factor and fibroblast growth factor-2) to the CNS [8]. This noninvasive delivery method targets therapeutics to the CNS, reducing systemic exposure and side effects. Thus, the intranasal route can be advantageous for delivery of many CNS drugs, including those that can cross the BBB upon systemic administration. Intranasal delivery of therapeutics to the CNS is rapid, occurring within minutes.

Routing drugs directly from the nasal cavity to the brain sidesteps the first-pass effect, during which metabolism in the liver, kidney filtration, and degradation greatly reduce the amount of active drug that eventually reaches the brain [9]. The duration and intensity of a drug's actions can also be affected by the degree to which it binds to proteins within blood plasma. The more drug that binds to protein, the less efficiently it can transport across the BBB [10]. Another concern with systemic administration is adverse systemic or even toxic side effects. Although ICV injection can deliver drugs directly to the brain, it is a highly invasive and risky procedure [11]. Insufflation of drugs through the nose is noninvasive, associated with few complications, and directs compounds directly to the CNS [12–14]. Because the effect is often reached within 5 min, nasal administration can be used as an alternative to oral administration. A variety of growth factors, hormones, neuropeptides, and stem cells can be delivered intranasally. Even large and/or charged therapeutics, which do not effectively cross the BBB, can be delivered via the intranasal method. Therefore, this route holds promise for treatment of many CNS-related diseases, including stroke [14]. Nevertheless, each drug must be tested for effects on the nasal mucosa, sense of smell, and immune system, as drugs will likely enter the nasal-associated lymphatics and deep cervical lymph nodes.

8.3 Intranasal Administration of Growth Factor Confers Protective Effects Against Ischemic Stroke

Numerous experimental studies have shown that a wide variety of peptide and protein therapeutics delivered by the intranasal route have the potential to treat ischemic stroke. In a study by Liu et al. [15] intranasal administration of insulin-like growth factor-1 (IGF-1, MW = 7.65 kDa) significantly reduced infarct size by 54% when given at 2 h after ischemia induction and 39% when given at 4 h. It also improved neurologic function. Intranasal delivery of recombinant human erythropoietin (rHu-EPO) was shown to reduce neurologic and cognitive deficits, as well as histologic damage in gerbils exposed to experimentally induced focal cerebral ischemia [16]. Fletcher et al. [17] demonstrated that EPO (MW = 30–34 kDa) plus IGF-1 penetrated the brain more efficiently when delivered by the intranasal route than when delivered by intravenous, intraperitoneal, and or subcutaneous injections. The intranasal combination of EPO and IGF-1 delivered 1 h after middle cerebral artery occlusion (MCAO) significantly reduced infarct volumes 24 h later and improved neurologic function up to 90 days later. Intranasal nerve growth factor (MW = 26.5 kDa) enhanced neurogenesis in the striatum and improved functional recovery when administered 24 h after MCAO [18]. Intranasal delivery of recombinant human VEGF (MW = 38.2 kDa) also reduced infarct volume, improved behavioral recovery, and enhanced angiogenesis following MCAO in rats [19]. In mice subjected to MCAO, intranasal delivery of TGF-β1 (MW = 25 kDa) reduced infarct

volume, increased neurogenesis in the SVZ, and improved functional recovery [20]. Ma et al. [21] reported improved neurologic function and reduced infarct volume in rats when basic fibroblast growth factor was delivered intranasally after cerebral ischemia/reperfusion. Rats that received intranasal basic fibroblast growth factor daily for 6 days beginning 1 day after MCAO also showed enhanced neurogenesis [22].

8.4 Intranasal Delivery of IL-17A Promotes Neurogenesis and Functional Recovery After Ischemic Stroke

The IL-17A family consists of several cytokines that participate in both acute and chronic inflammatory responses [23]. IL-17A is the most widely investigated cytokine of this family, and its production has been mainly attributed to T helper 17 (Th17) cells [23]. Recent studies have revealed that IL-17A is mainly produced by gamma delta (γδ) T cells in the acute phase of ischemic stroke [24, 25]. As a linkage between innate and adaptive immunity, IL-17A secreted from γδ T cells plays detrimental roles in acute ischemic stroke [24, 26]. Evidence has shown that IFN-γ produced by $CD4^+$ T cells induces TNF-α production in macrophages, whereas IL-17A secreted by γδ T cells triggers neutrophil recruitment to the infarcted hemisphere. The synergistic effect of TNF-α and IL-17A on astrocytes enhanced secretion of neutrophil-attracting chemokine CXCL-1 [25]. CXCL-1 binds to its receptor CXCR-2, resulting in the recruitment of neutrophils to the infarcted site, thus amplifying the inflammatory response and contributing to tissue damage [27]. Application of an IL-17A-blocking antibody after ischemic stroke induction decreases infarct size and improves neurologic outcome in the murine model. Additionally, IL-17A-positive lymphocytes were detected in postmortem brain tissue of patients who had experienced a stroke, suggesting that this aspect of the inflammatory cascade also occurs in the human brain [24]. In our previous study, we found that IL-17A from reactive astrocytes maintained and augmented the survival and neuronal differentiation of neural precursor cells in the SVZ during stroke recovery and subsequent synaptogenesis and spontaneous recovery through the p38 mitogen-activated protein kinase (MAPK)/calpain 1 signaling pathway [28]. We have also shown that pro-angiogenesis effects of IL-17A are involved in post-stroke functional recovery [29]. Therefore, although IL-17A is well-known for its damaging role in acute stroke, it might be an essential mediator for ischemia-induced neurorepair and spontaneous recovery. Our findings reveal a previously unrecognized role for IL-17A in neurogenesis, angiogenesis, and subsequent synaptogenesis and long-term functional recovery after ischemic stroke (Fig. 8.1) [28]. Importantly, these results indicate that IL-17A may have a biphasic role in different phases of ischemic stroke.

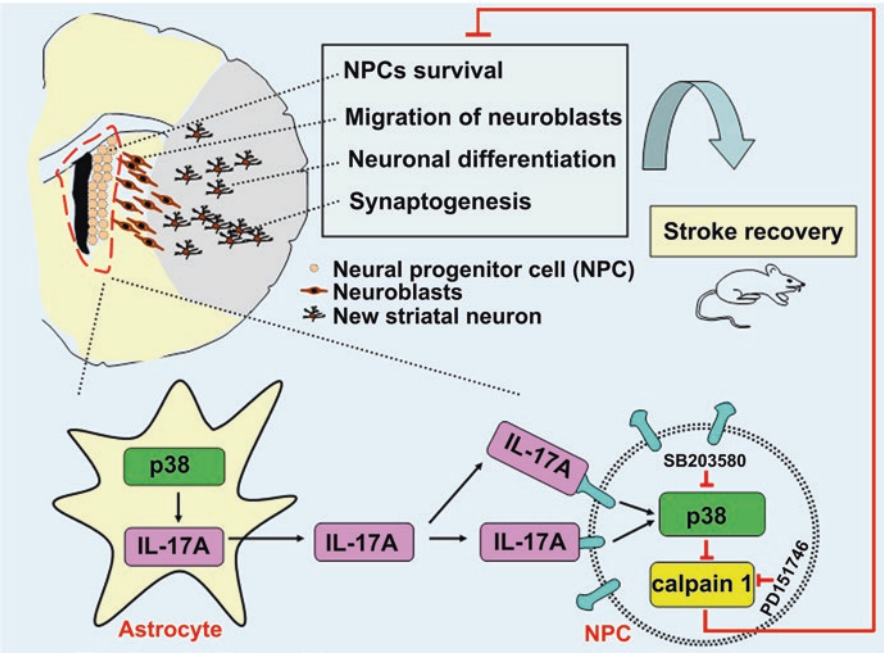

Fig. 8.1 Intranasal delivery of IL-17A promotes functional recovery by enhancing neural progenitor cell (NPC) survival, neuroblast migration, neuronal differentiation, and synaptogenesis through the p38 MAPK/calpain 1 signaling pathway

8.5 The Dual Effects of IL-17A in Different Stages of Ischemic Stroke

Signals that are deleterious during the acute stage of stroke may play beneficial roles during the recovery phase. Many reports in the literature based on cell and animal models suggest that N-methyl-D-aspartate (NMDA) receptor, the matrix metalloproteinase (MMP) family, and high-mobility-group-box-1 (HMGB1) worsen acute brain injury after stroke. However, recent studies suggest that they all could promote endogenous neurogenesis in the later phases of stroke recovery [30–33]. Another example of the biphasic nature of molecular signals involves nitric oxide (NO). Accumulating data indicate that NO is deleterious when large amounts are produced by uncontrolled neuronal or inducible nitric oxide synthase isoforms [34, 35]. Alternatively, however, NO promotes angiogenic sprouting [36]. Angiogenesis is an important feature of the peri-infarct cortex during stroke recovery [37, 38]. Therefore, some molecular signals may have biphasic roles after stroke.

Similar patterns may emerge when one looks at other stroke injury mechanisms. Among various immune cytokines, we focused on IL-17A because of two recently proposed ideas. First is the suggestion that IL-17A can exert both deleterious and beneficial effects in neuroinflammation [24, 39, 40]. Second, the crosstalk between

central reactive astrocytes and precursor cells during stroke recovery supports neurovascular remodeling and functional recovery [33]. In neuroinflammatory diseases, IL-17A is specifically expressed in reactive astrocytes [40, 41]. As expected, we showed that IL-17A from reactive astrocytes promoted neurorepair and long-term functional recovery [28]. Therefore, our results indicate that IL-17A may have a biphasic role in ischemic stroke. IL-17A from γδ T cells may worsen acute brain injury in the acute stage of stroke [24], whereas IL-17A from astrocytes may promote neurogenesis, angiogenesis, and functional recovery.

8.6 Intranasal Application of IL-17A After Cerebral Ischemia

In consideration of the possible detrimental effects of IL-17A in various peripheral tissues and organs during stroke recovery, the perilesional accumulation of IL-17A in the brain seems to be the key to its neurogenic effects after ischemic stroke. Therefore, we chose the intranasal delivery route for our previous study [28]. We used a sterile 26-G Hamilton microsyringe (80330; Hamilton Company, Reno, NV) to intranasally administer 2 μL drops of recombinant mouse IL-17A (rIL-17A) diluted in PBS containing 0.1% albumin (0.1 μg/μL) or its vehicle (PBS containing 0.1% albumin) to alternating nostrils, with a 2-min interval between applications. Drops were placed at the opening of the nostril, allowing the mouse to inhale each drop into the nasal cavity. A total of 10 μL of solution, containing 1 μg rIL-17A, was delivered over the course of 5 min. The administration of rIL-17A (or vehicle) was repeated every 24 h for 2 weeks starting at 14 days post-ischemia.

We should note that, although IL-17A delivered through the nasal route may promote neurorepair and functional recovery, systemic (intravenous and intraperitoneal, etc.) administration of IL-17A may confer detrimental effects on peripheral tissues and organs because of its proinflammatory effects. Whether intranasal IL-17A can reach systemic circulation and confer detrimental effects in the peripheral system remains unknown.

8.7 Intranasal Application of EPO After Cerebral Ischemia

Another example of a compound that can be administered nasally is EPO. rHu-EPO has been tested in experimental stroke models, but its hematopoietic effect, as well as alterations in platelet function and hemostasis, might elicit potential adverse effects if used systemically in patients [42]. The main advantages of intranasal administration of EPO include the lack of hematopoietic activity and the lower doses required. Evidence has shown that intranasal delivery of rHu-EPO confers long-term neuroprotection without side effects on the hematopoietic system [43].

8.8 Conclusion

Thus, intranasal administration could be the most promising, efficient, and noninvasive route for delivering therapeutic substances directly to brain for the treatment of ischemic stroke without invasiveness or systemic adverse effects. It also could increase patient comfort and compliance. Intranasal delivery of IL-17A or other compounds may hold promise for promoting post-stroke neurovascular repair and long-term functional recovery.

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